

Earl
SapI

TTTAAAGGAAGATACACCTCTCTTATGGNAATTGGAAGACCTTGATAAATGGATGAACAGCATGATAGAAATGCCGACTTTGAATGTTTACCTTCTGAAGGAGAGAGAGAAATCAAAATCACACCCAGTGCAGTGAATC
AATACTCTTCTATGTGGAGACAATACCTTTAACTTTACCTTCTGAACTATTACCTACTTTCGACTTATCTTACGGCTGAAACTTACAAATGGTTGGAACCTCTCTCTCTTACTTGTAGTTGGTTTCACTGTCCTAG
...
E E E D T P S V M E I E M E E L D K W M N S M N R N A D F E C L P T L K E E K E S N H N P S D S E S

AlwNI
Bsu36I

BbvCI
Bpu10I

BamHI

(SEQ ID NO. 2)

TTTAAACCTGAATGGCGCTCATGCTTTTCCAAGAGAGCAGCCCTCGAGGGAGTCTGCTGAGGCTGCCAACAGGATCC
TTTGGACTTACCGCGAGTACAAAGGTTCTCTTCGTCGGGAGCTCCCTCAGACGAGCTCCGAGCGGTTGTCTTAGG 676

R1 Primer

FIG. 1 *con't*

FLJ32028 protein with N-terminal HA Tag

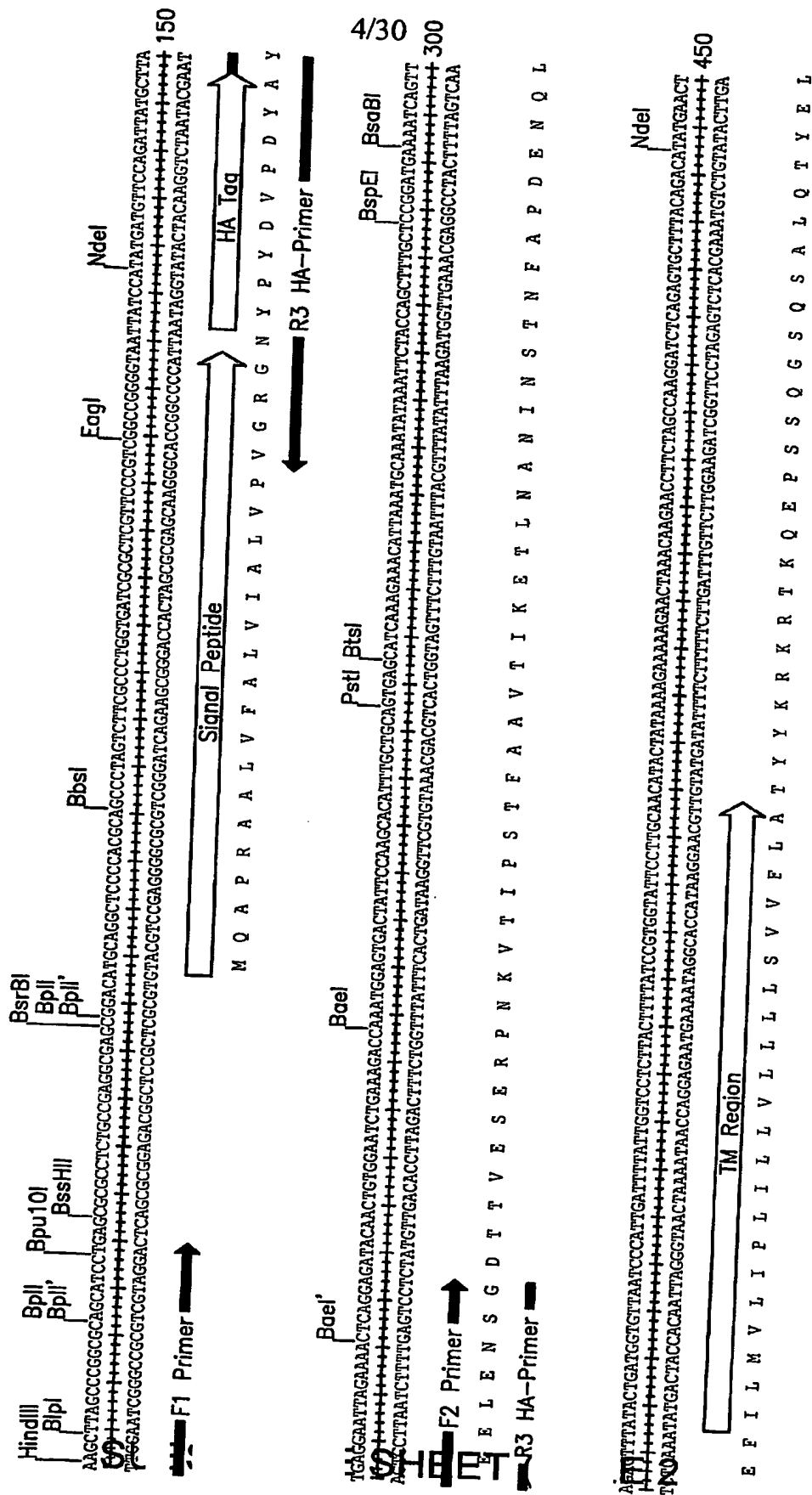


FIG. 3

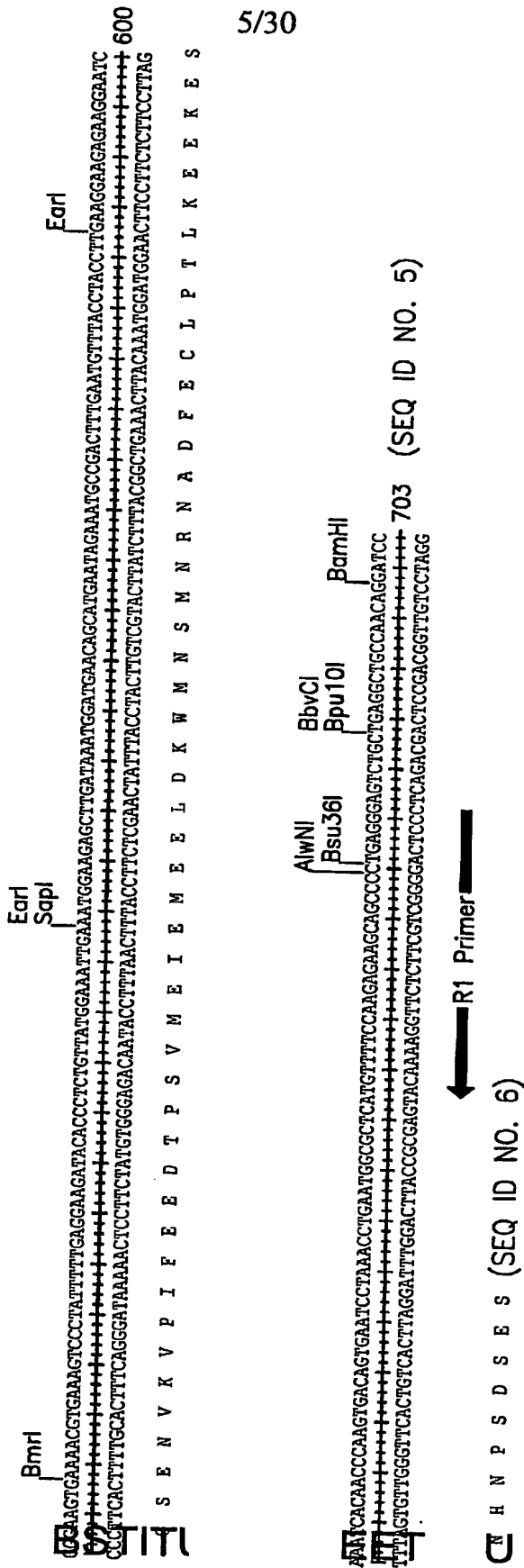


FIG. 3 con't

Ndel: GAATTC
 BmrI: GATATC

L I L L V L L L L S V V F L A T Y Y K R K R T K Q E P S S Q G S Q S A L Q T Y E L G S E N V K V P I

TM Region

FIG. 4

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600

637 (SEQ ID NO. 7)

Bsu36I Bpu10I BamHI

R2-HA Primer

HA-Taq

600

637 (SEQ ID NO. 7)

R2-HA Primer

HA-Taq

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FIG. 4 con't

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FACs Analysis of Transfected 293-EBNA Cells

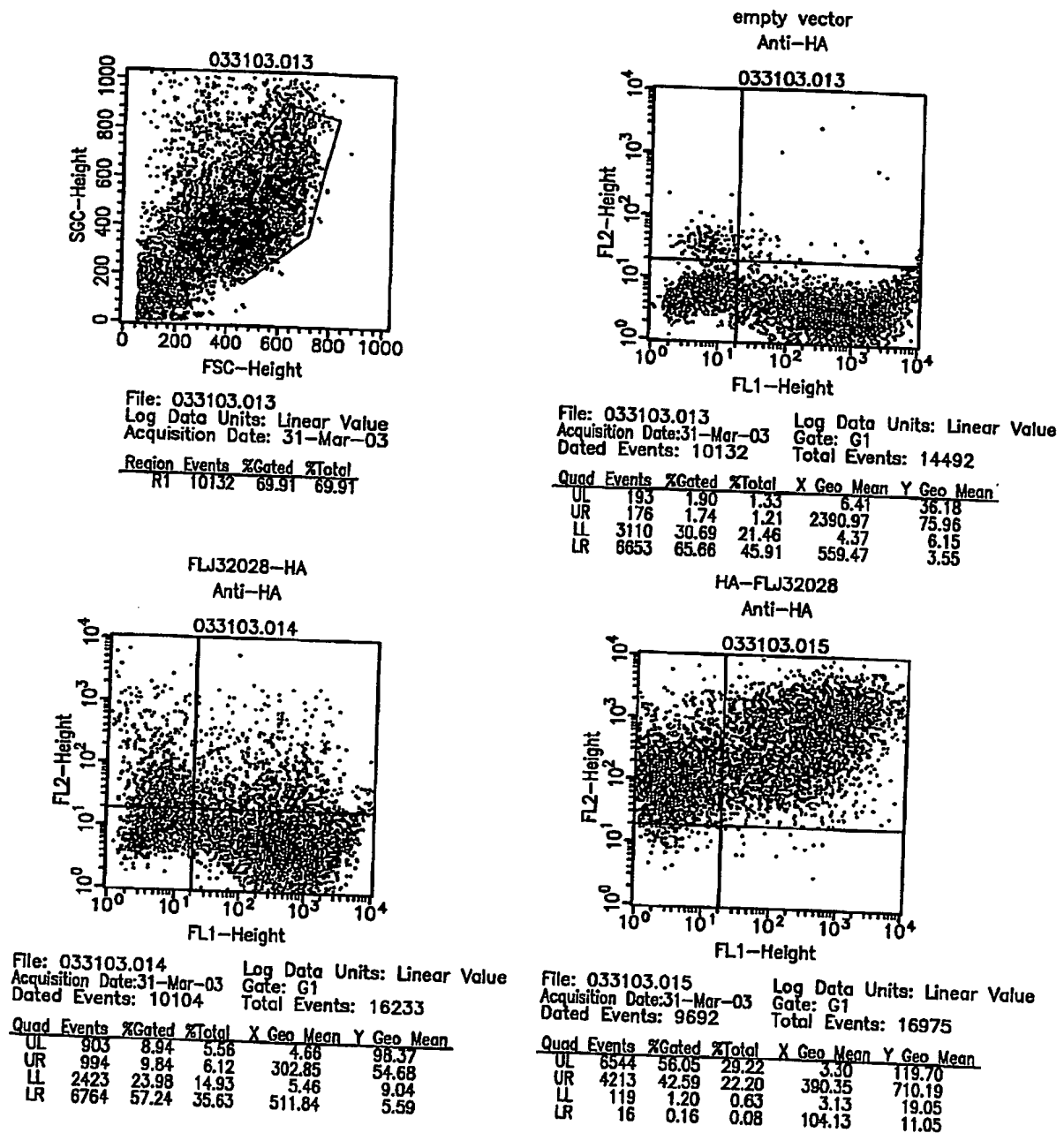


FIG. 5

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Anti-HA Western Blot of Transfected 293-EBNA Cells

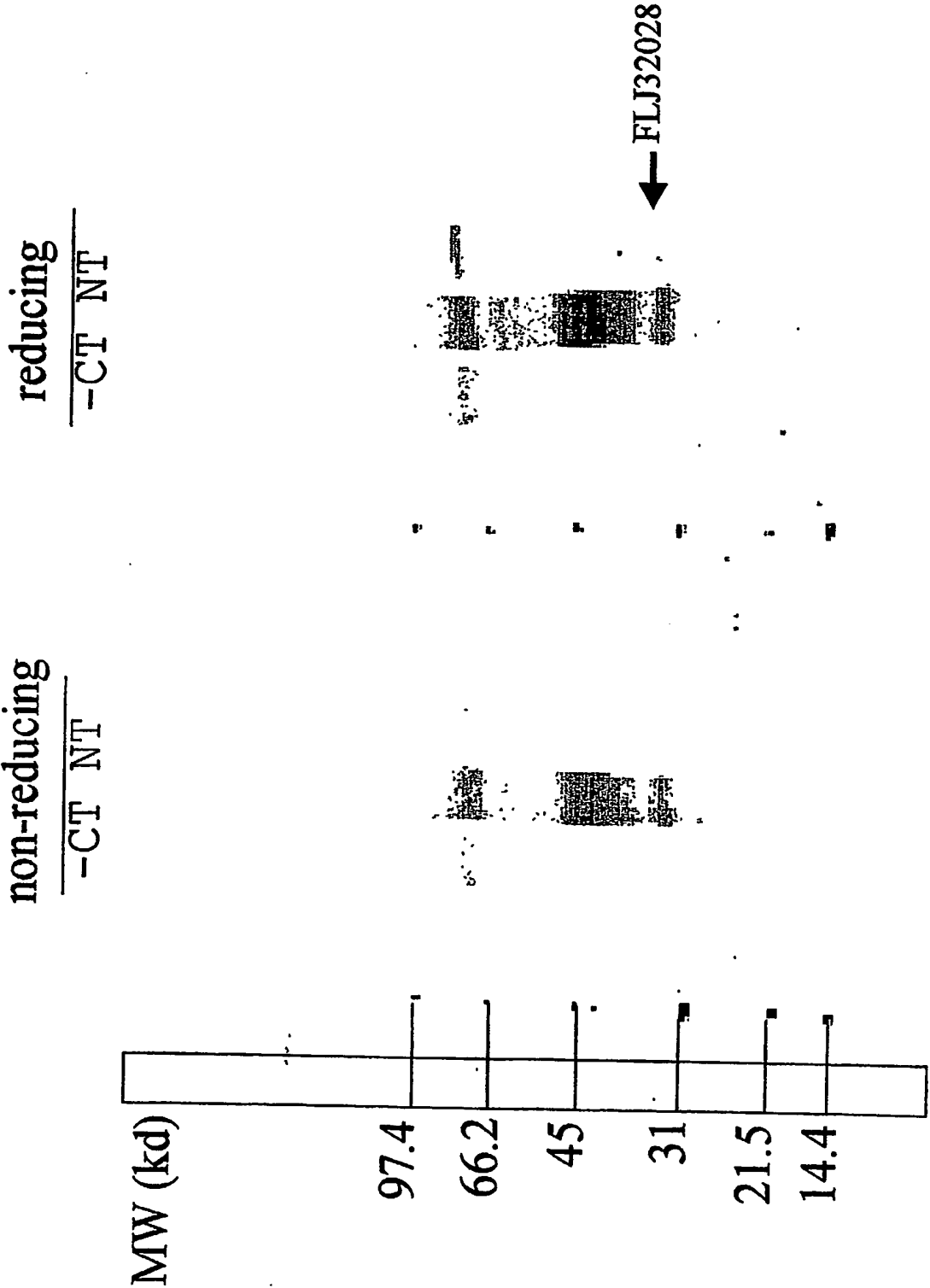


FIG. 6

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FLJ32028 polynucleotide sequence (SEQ. ID No. 2):

AGCCCGGCGCAGCATCCTGAGCGCGCCTCTGCCGAGGCGAGCGGACATG
CAGGCTCCCCGCGCAGCCCTAGTCTTCGCCCTGGTGATCGCGCTCGTTCC
CGTCGGCCGGGGTAATTATGAGGAATTAGAAAACCTCAGGAGATACAACTGT
GGAATCTGAAAGACCAAATAAAGTGACTATTCCAAGCACATTTGCTGCAGTG
ACCATCAAAGAAACATTAAATGCAAATATAAATTCTACCAACTTTGCTCCGGA
TGAAAATCAGTTAGAGTTTATACTGATGGTGTTAATCCCATTTGATTTTATTGG
TCCTCTTACTTTTATCCGTGGTATTCTTGCAACATACTATAAAAGAAAAAGA
ACTAACAAGAACCTTCTAGCCAAGGATCTCAGAGTGCTTTACAGACATATGA
ACTGGGAAGTGAAAACGTGAAAGTCCCTATTTTTGAGGAAGATACACCCTCT
GTTATGGAAATTGAAATGGAAGAGCTTGATAAATGGATGAACAGCATGAATA
GAAATGCCGACTTTGAATGTTTACCTACCTTGAAGGAAGAGAAGGAATCAAA
TCACAACCCAAGTGACAGTGAATCCTAAACCTGAATGGCGCTCATGTTTTCC
AAGAGAAGCAGCCCCTGAGGGAGTCTGCTGAGGCTGCCAACA

FIG. 7

FLJ32028 polypeptide sequence (SEQ. ID No. 1):

MQAPRAALVFALVIALVPVGRGNYEELNSGDTTVESERPKNKVTIPSTFAAVTIK
TLNANINSTNFAPDENQLEFILMVLIPLILLVLLLLSVFLATYYKRKRTKQEPSSQ
GSQSALQTYELGSENVKVPIFEEDTPSVMEIEMEELDKWMNSMNRNADFECLP
TLKEEKESNHNPSDSES

FIG. 8

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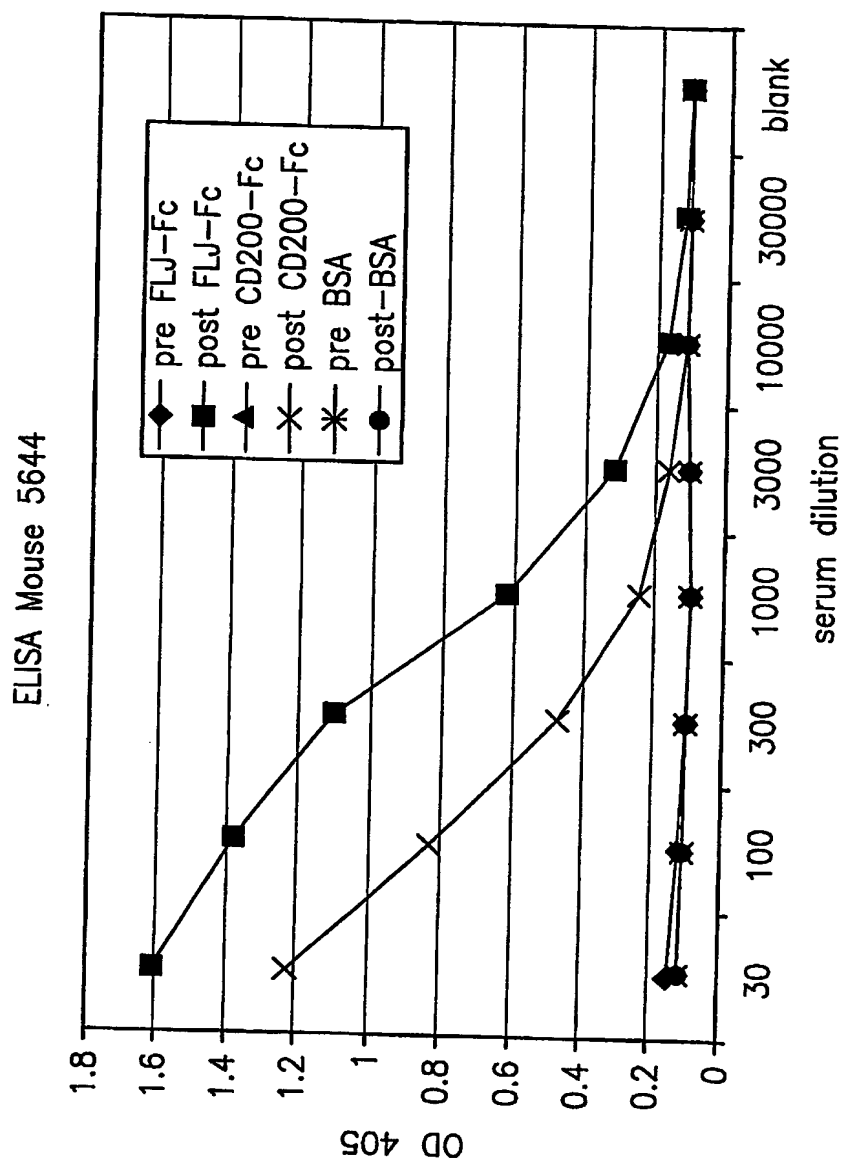
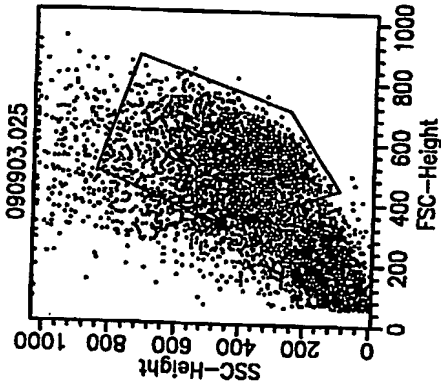


FIG. 9A

Mouse 5644



Key	Name	Parameter	Gate
▨	090903.025	FL2-H	G1 pre 1:30/FLJ
—	090903.031	FL2-H	G1 post 1:30/FLJ
—	090903.037	FL2-H	G1 post 1:100/FLJ
—	090903.043	FL2-H	G1 post 1:300/FLJ
—	090903.007	FL2-H	G1 post 1:30/mock
—	090903.013	FL2-H	G1 post 1:100/mock
—	090903.019	FL2-H	G1 post 1:300/mock

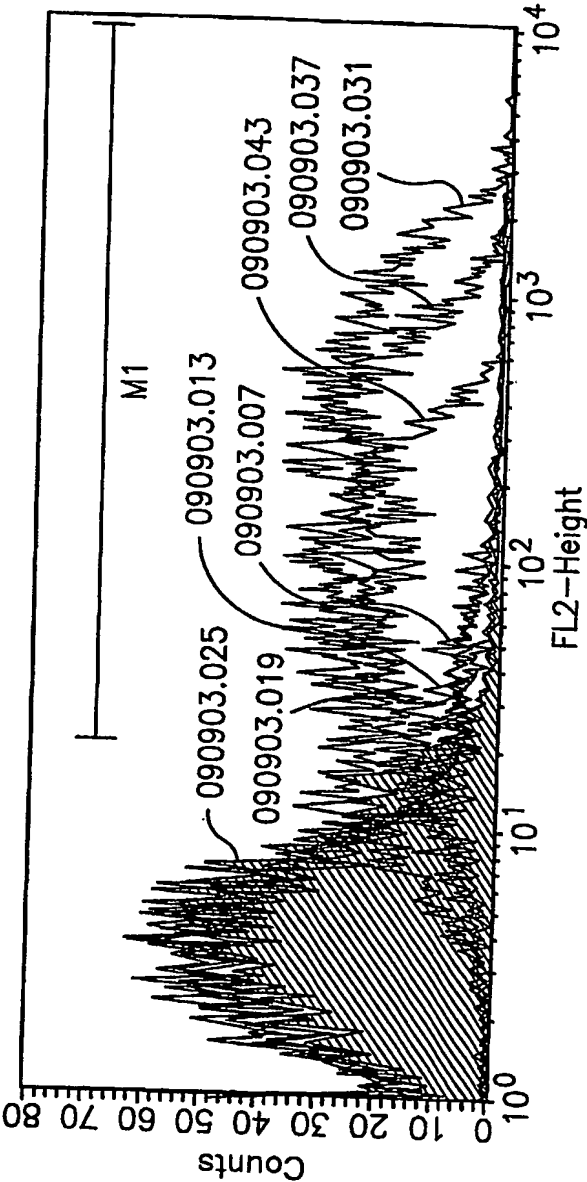


FIG. 9B

ELISA Mouse 5640

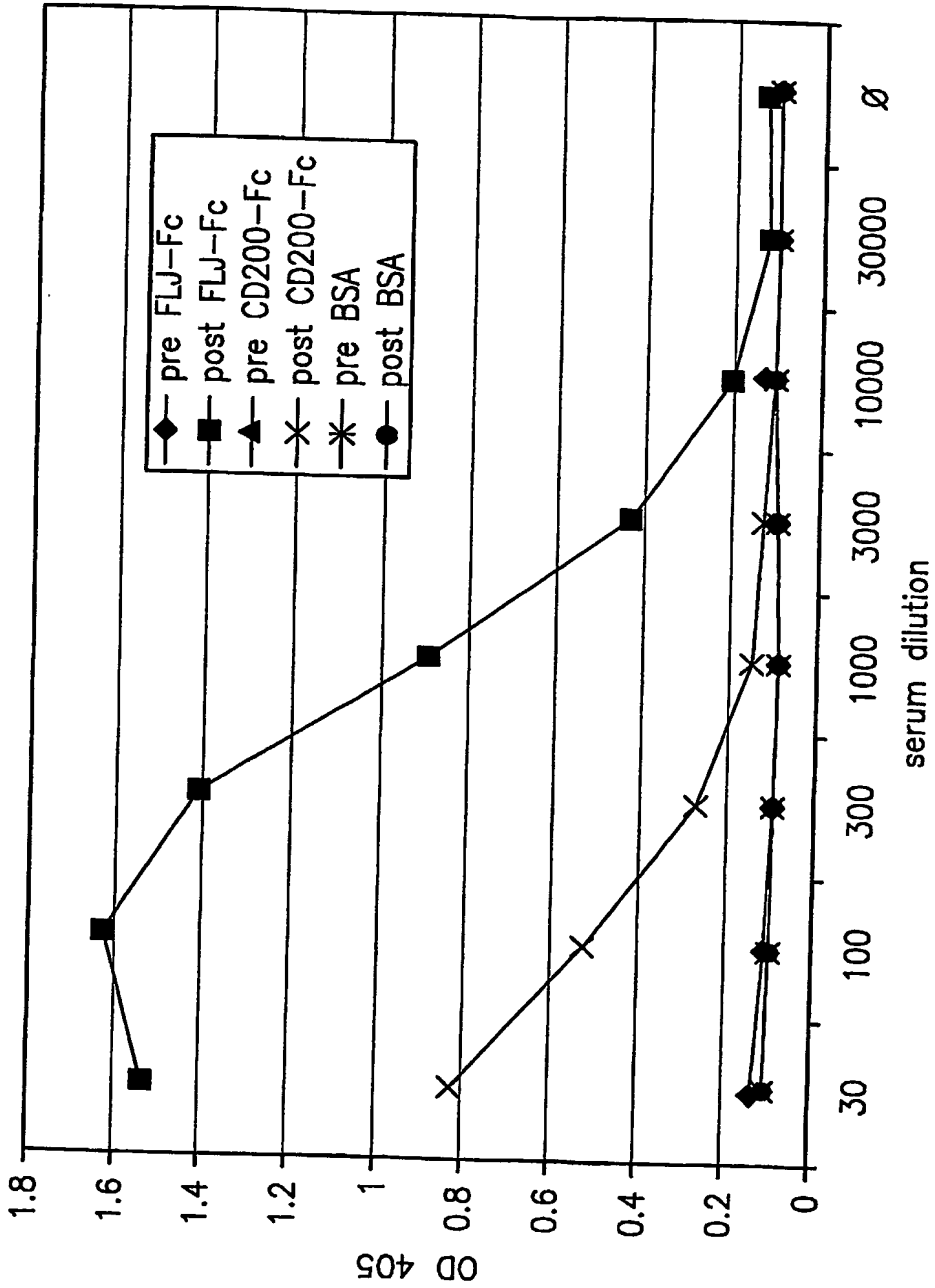
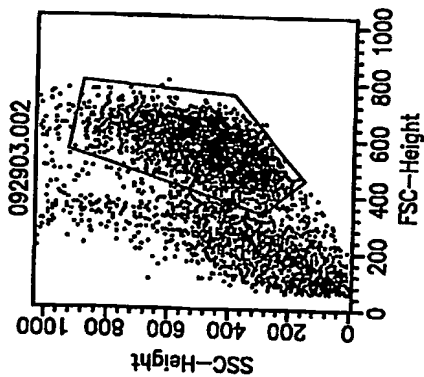


FIG. 10A

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Mouse 5640



Key	Name	Parameter	Gate
▨	092903.016	FL2-H	G1 pre 1:30/FLJ
—	092903.020	FL2-H	G1 post 1:30/FLJ
—	092903.023	FL2-H	G1 post 1:100/FLJ
—	092903.026	FL2-H	G1 post 1:300/FLJ
—	092903.006	FL2-H	G1 post 1:30/mock
—	092903.009	FL2-H	G1 post 1:100/mock
—	092903.012	FL2-H	G1 post 1:300/mock

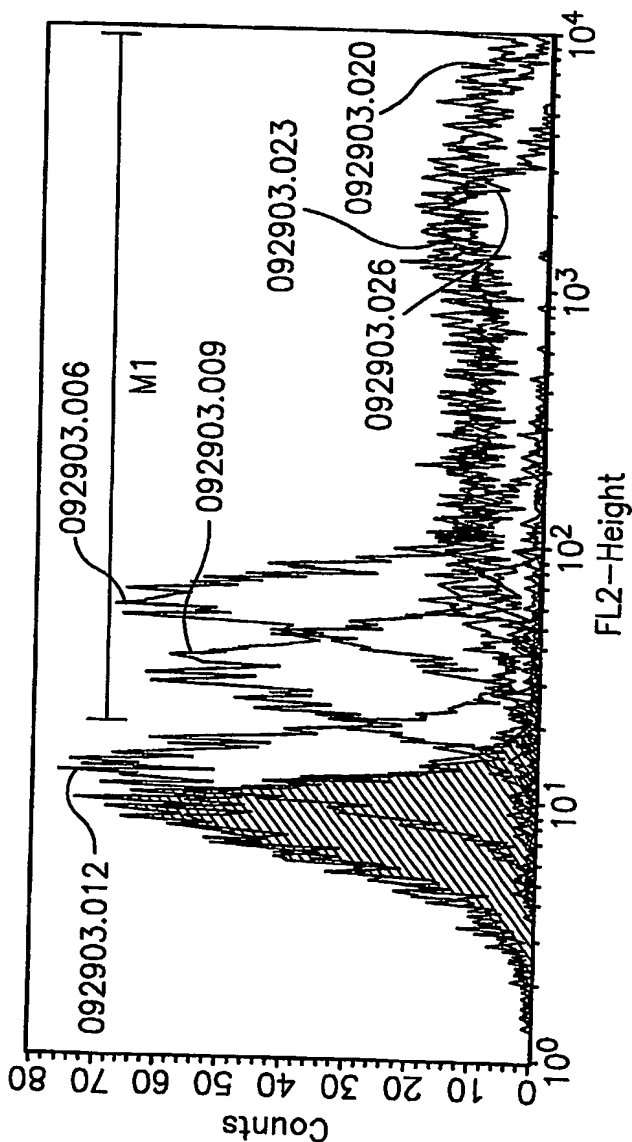
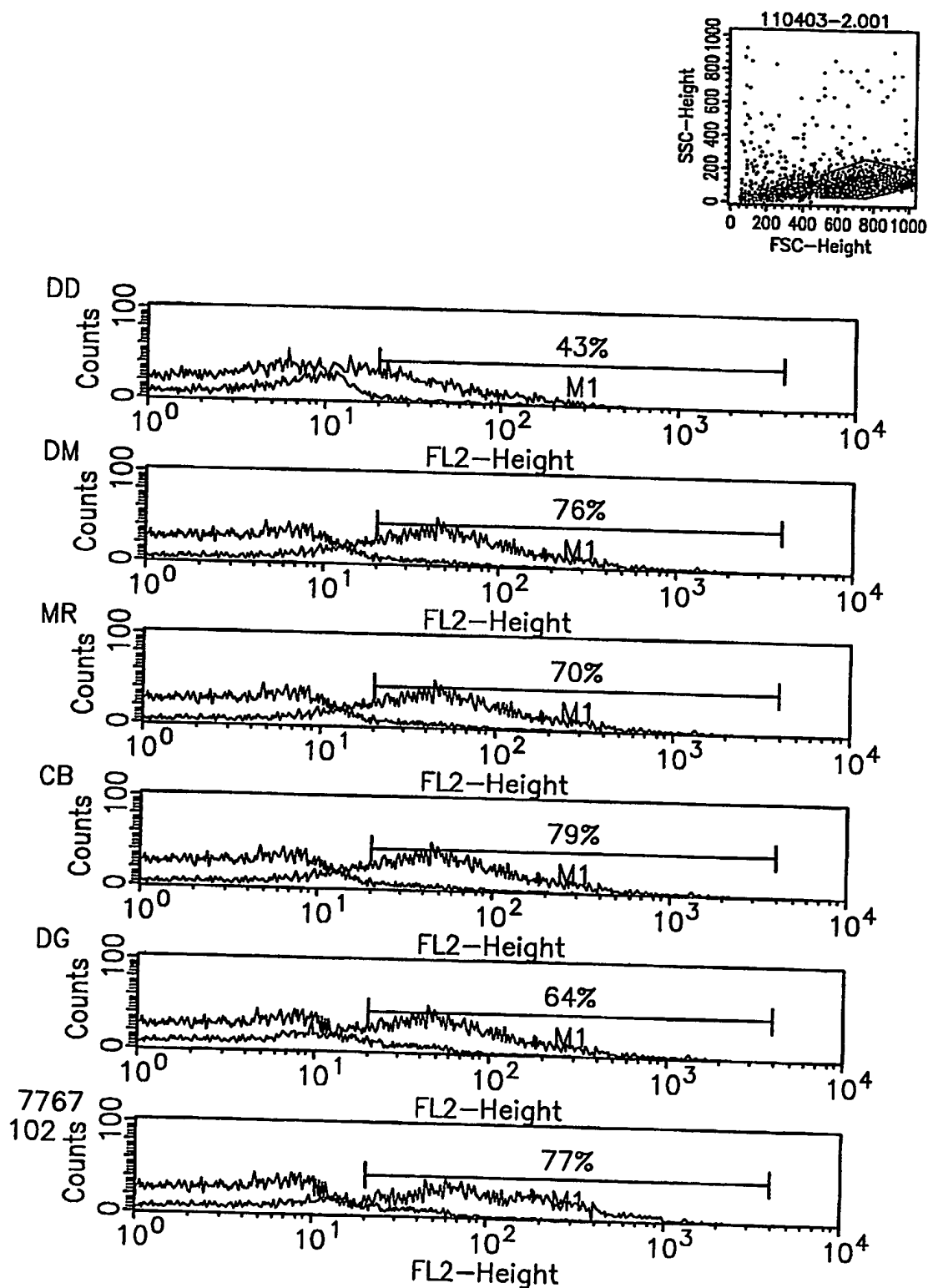


FIG. 10B

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**FIG. 11**

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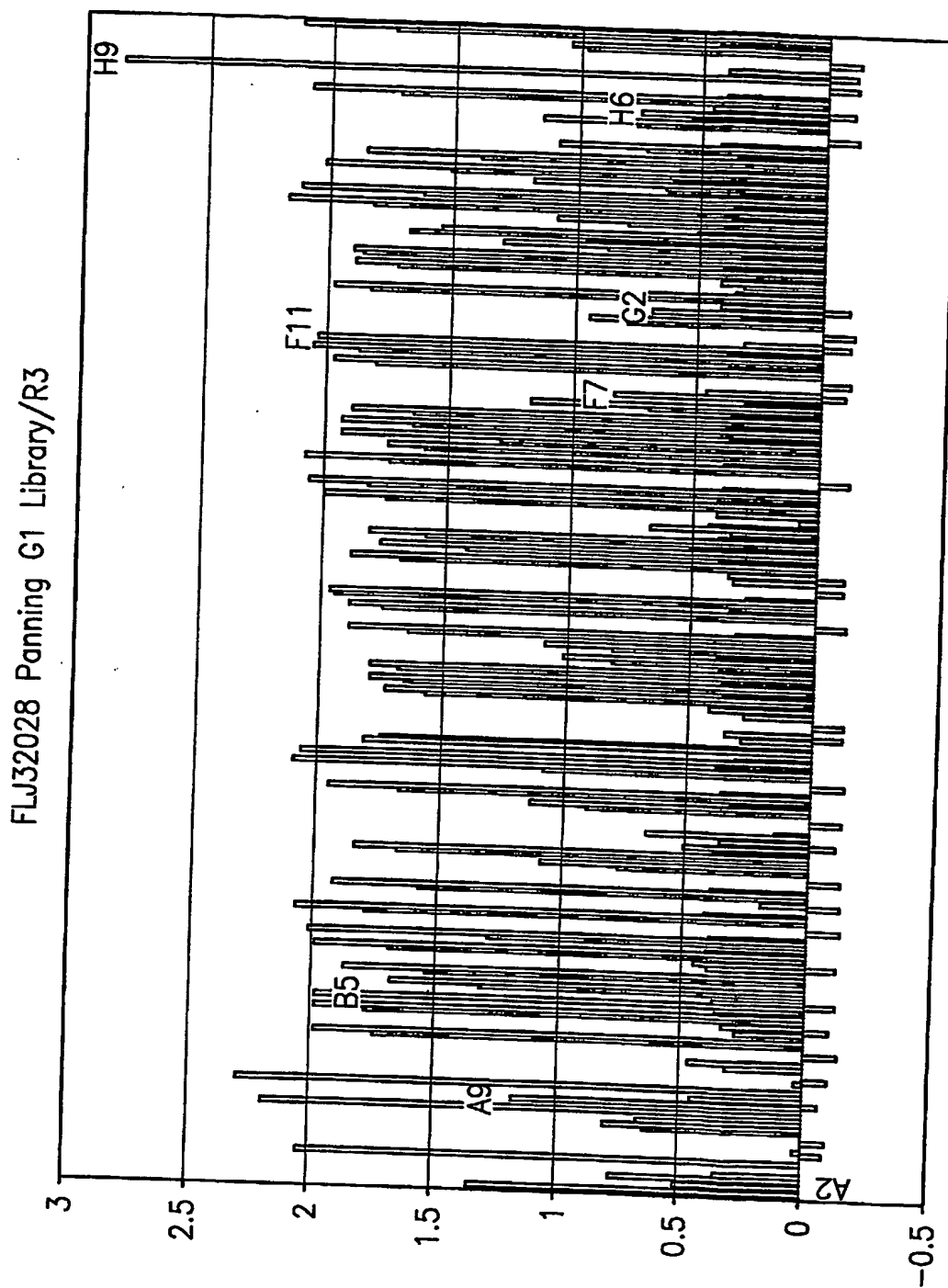


FIG. 12

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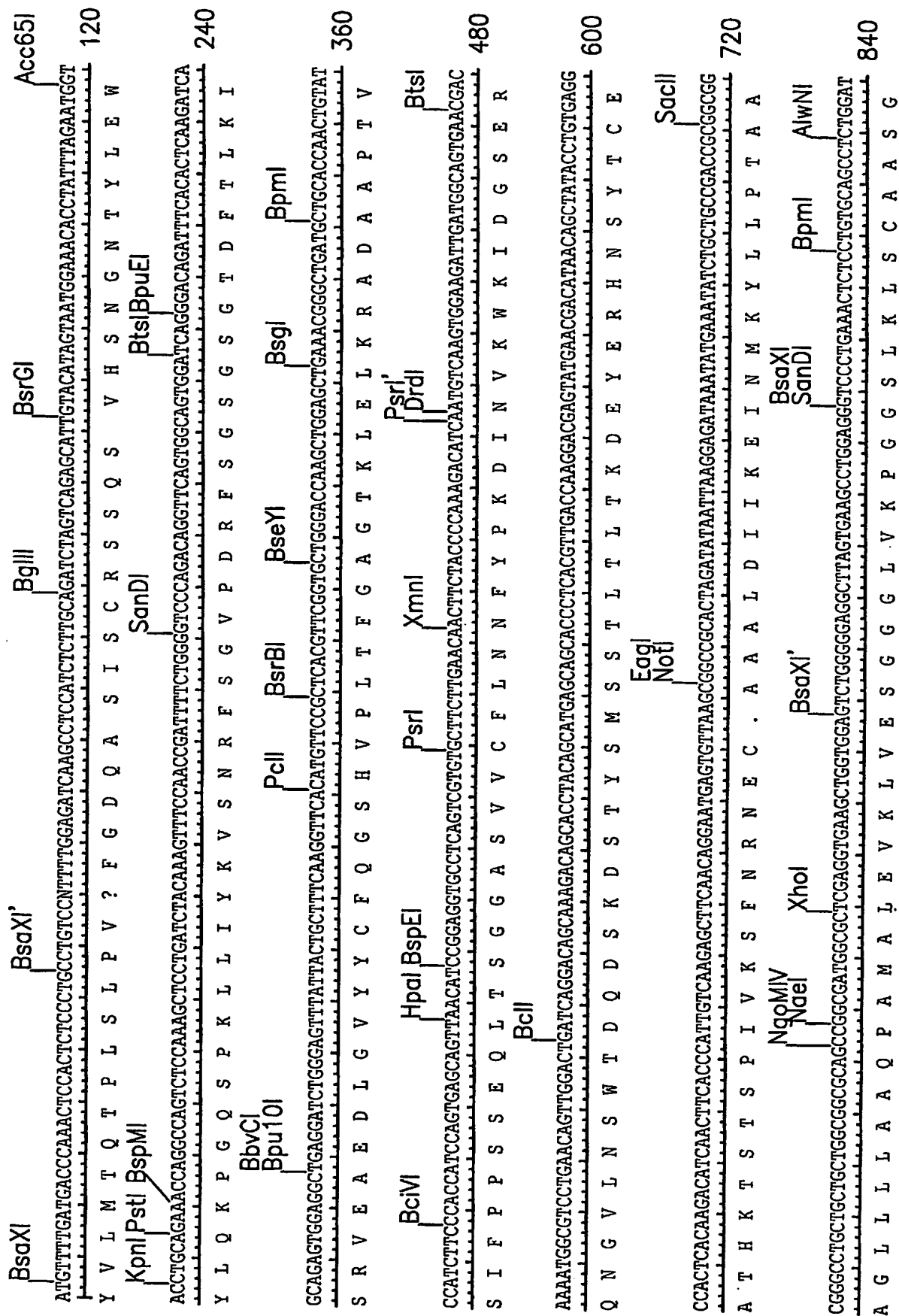


FIG. 13



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FIG. 14

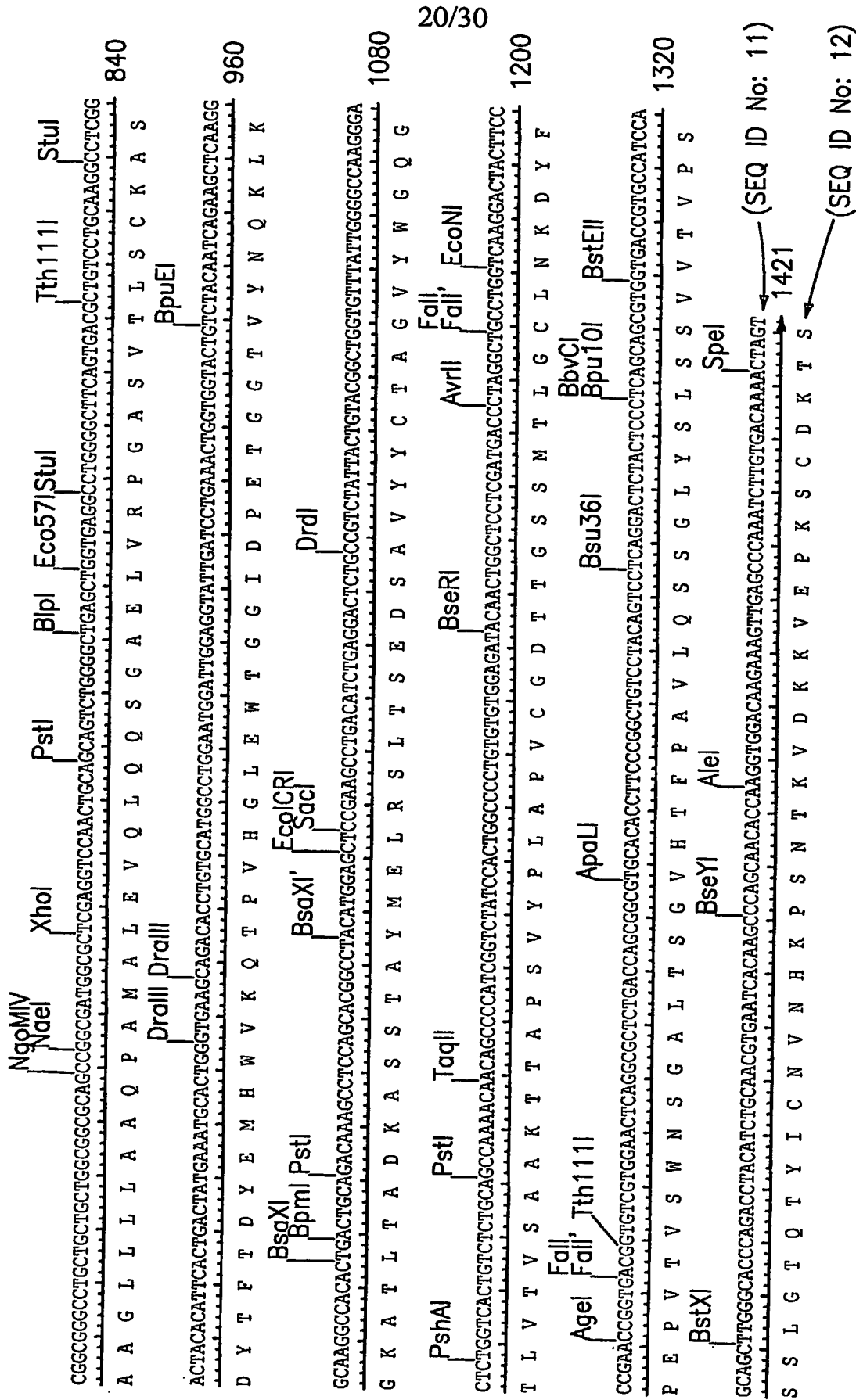


FIG. 14 (Cont.)

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VL amino acid sequences of FLJ32028-specific IgG1 kappa
clones from 5644 library

Clone	FR1	CDR1	FR2	CDR2
11F	DIQMTQTSSLSASLGDRVTISCRTS	QDISN-----Y LNWYQQKPDGTVKVLII	YTS	
11G	..V...AEL·SPVTS·ES·S...S·	KSLLYK-DGKT·	...L·R·GQSPQL...	FM·
6H	..V...SP...AV·V·EK·M·KS·	·SLLYSSNQKN·	·A...·GQSP·L...·	WA·
9H	..V·S·SP...AV·V·EK·M·KS·	·SLLYSSNQKN·	·A...·GQSP·L...·	WA·
9A	·VV...PL·PV...QAS...S·	·S·VHS-NGNT·	·E·L...GQSP·L...·	KV·
2G	·VV...PL·PV...QAS...S·	·S·VHS-NGNT·	·E·L...GQSP·L...·	KV·
7F	·VL...PL·PV...QAS...S·	·S·VHS-NGNT·	·E·L...GQSP·L...·	KV·

ELISA ODs:			FR4		CDR3		FR4		ELISA ODs:		SEQ ID NO:
			FR3						FLJ/	Fc/ Fab	
RLHSGVPSRFGSGGTDYSLTINNLEQED	IATYFC	QQGNTLPFTFGSG	TKLEIKR						2.14/0.12/0.46		(SEQ ID NO: 13)
TRAP·SD...I...FT·E·SRVKA·	VG·Y·	·LVEY·L...A·	...L...						1.98/0.14/0.41		(SEQ ID NO: 14)
TRE...D...T...FT...SSVKA·	L·V·Y·	·YYSY·L...A·	...L...						0.86/0.12/0.61		(SEQ ID NO: 15)
ARG...D...T...FT...SSVKA·	L·V·Y·	·YYSY·L·I·A·	...L...						2.95/0.11/0.56		(SEQ ID NO: 16)
NRF...D...FT·K·SRV·A·	LGV·Y·	F·SHV·L...A·	...L...						1.30/0.17/0.61		(SEQ ID NO: 17)
NRF...D...FT·K·SRV·A·	LGV·Y·	F·SHV·L...A·	...L...						0.80/0.12/0.57		(SEQ ID NO: 17)
NRF...D...FT·K·SRV·A·	LGV·Y·	F·SHV·L...A·	...L...						0.95/0.13/0.62		(SEQ ID NO: 17)

FR: framework region; CDR: complementarity determining region. Dots indicate identities with the clone 11F sequence. Dashes indicate gaps in the alignment. ELISA ODs are given for binding of the phage antibody clones to FLJ32028-Fc fusion protein(FLJ), to negative control Fc fusion protein(Fc), and to Anti-F(ab')₂ (Fab).

FIG. 15

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VH amino acid sequences of FLJ32028-specific IgG1 kappa
clones from 5644 library

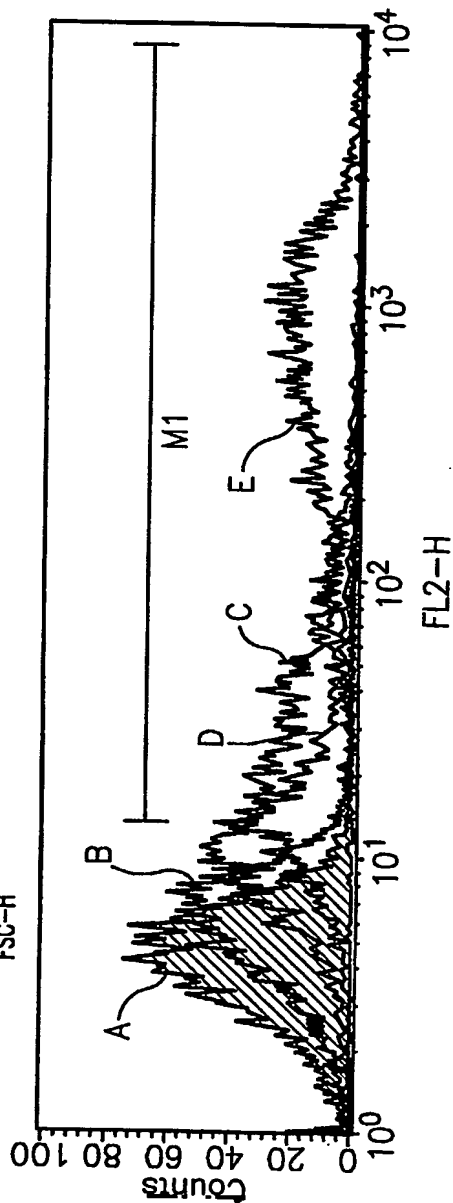
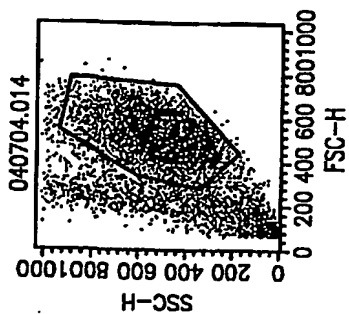
Clone	FR1	CDR1	FR2	CDR2
11F	LEVQLQQSGAELVRPGASVTLCKAS	GYTFTDYEMH WVKQTPVHGLEWIG	GIDPEIGGTVYNQKFKG	
11G	D.....T.....	L..
5BV.....Q..D.....S..A.....
6HK.....K.....T..	..FNIK..TYINR..EQ.....	R..ANN..N..DP..Q..
9HF.....K.....TG..	..FNIK..TY..NI..R..EQ.....	M..AN..N..Q..DP..Q..
9A	...K..VE..GG..K..G..LK..A..	..F..S..A..SR..EKR.....VA	S..S..SG..T..Y..LDSV..
2G	..AK..VE..GG..K..G..LK..A..	..F..S..A..SR..EKR.....VA	S..S..SG..T..Y..LDSV..
7F	...K..VE..GG..K..G..LK..A..	..F..S..A..SR..EKR.....VA	S..S..SG..T..Y..LDSV..

ELISA ODs:			FLJ/ Fc/ Fab	
FR3	CDR3	FR4		
KATLTADKSSGTAYMELRSLTSEDSAVYYCTS	F---AY WQGGTLVTVA		2.14/0.12/0.46	(SEQ ID NO: 18)
.....A..S.....	A G---V.....		1.98/0.14/0.41	(SEQ ID NO: 19)
.....R.....	A G---ADF..	1.84/0.11/0.53	(SEQ ID NO: 20)
...I...TP..N...LQ..S.....TD.....V..	GGYFD.....TL...S	0.86/0.12/0.61	(SEQ ID NO: 21)
...I...T..N...LQ..S.....T.....	GGYFD.....TL...S	2.95/0.11/0.56	(SEQ ID NO: 22)
RF..ISR..NARNIL..LQMS..R...T..M...VR	S-ETN.....TL...S	1.30/0.17/0.61	(SEQ ID NO: 23)
RF..ISR..NARNIL..LQMS..R...T..M...VR	S-ETN.....TL...S	0.80/0.12/0.57	(SEQ ID NO: 24)
RF..ISR..NARNIL..LQMS..R...T..M...VR	S-ETN.....TL...S	0.95/0.13/0.62	(SEQ ID NO: 23)

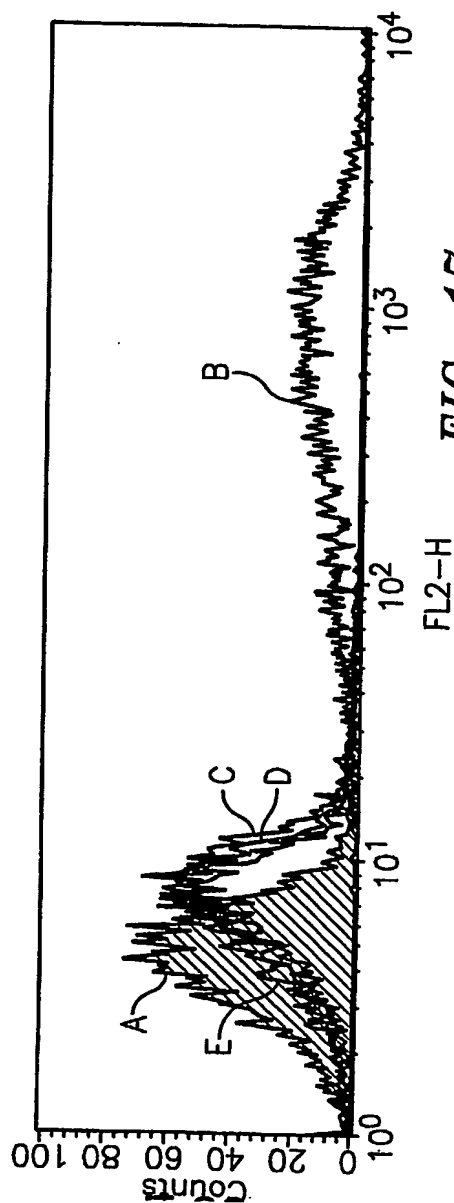
FR: framework region; CDR: complementarity determining region. Dots indicate identities with the clone 11F sequence. Dashes indicate gaps in the alignment. ELISA ODs are given for binding of the phage antibody clones to FLJ32028-Fc fusion protein(FLJ), to negative control Fc fusion protein(Fc), and to Anti-F(ab')₂ (Fab).

FIG. 16

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Key	Name	Parameter	Gat
▨	040704.014	FL2-H	G1 A
—	040704.022	FL2-H	G1 B
—	040704.020	FL2-H	G1 C
—	040704.024	FL2-H	G1 D
—	040704.019	FL2-H	G1 E



Key	Name	Parameter	Gat
▨	040704.014	FL2-H	G1 A
—	040704.018	FL2-H	G1 B
—	040704.021	FL2-H	G1 C
—	040704.017	FL2-H	G1 D
—	040704.023	FL2-H	G1 E

FIG. 17

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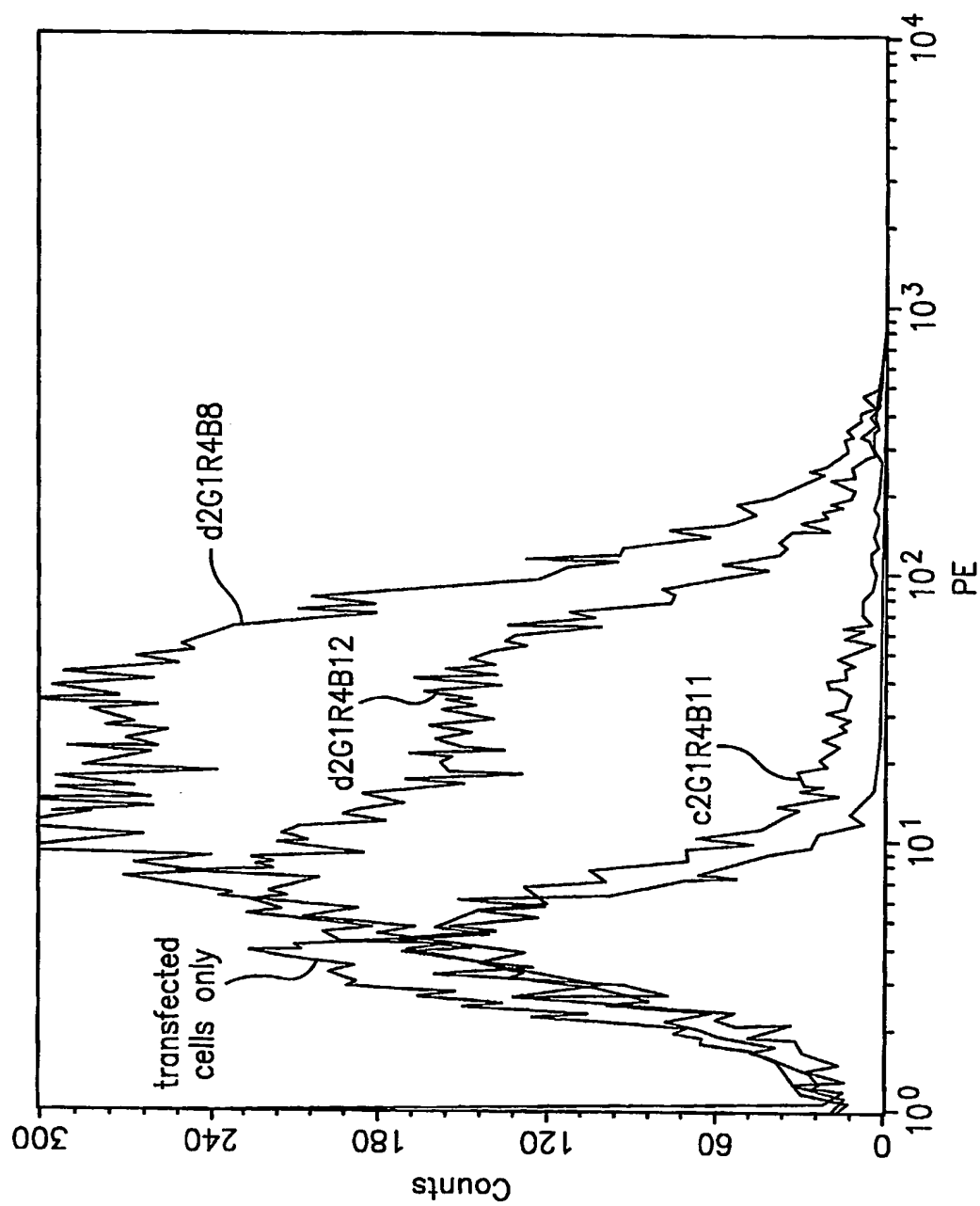


FIG. 18A

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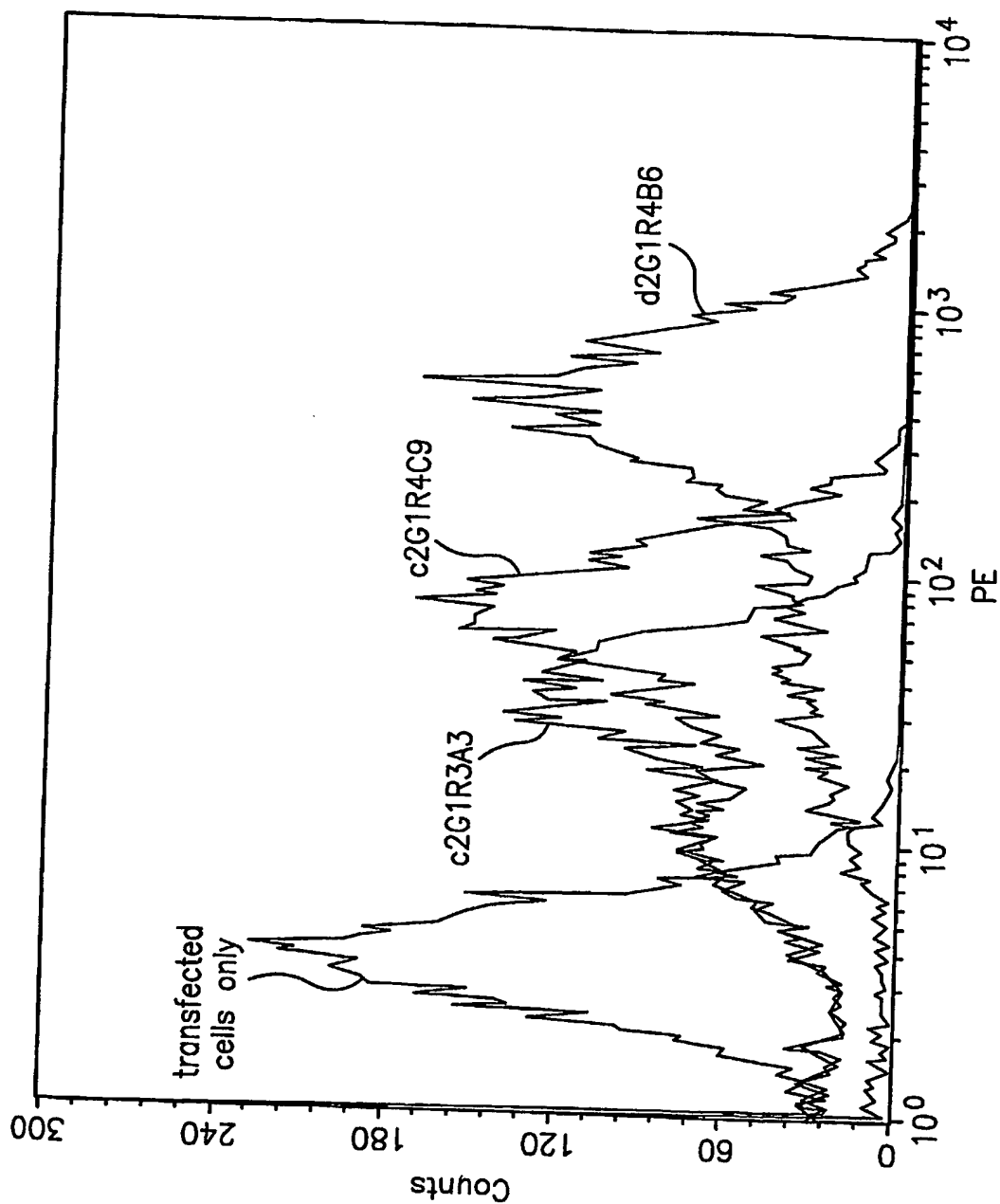


FIG. 18B

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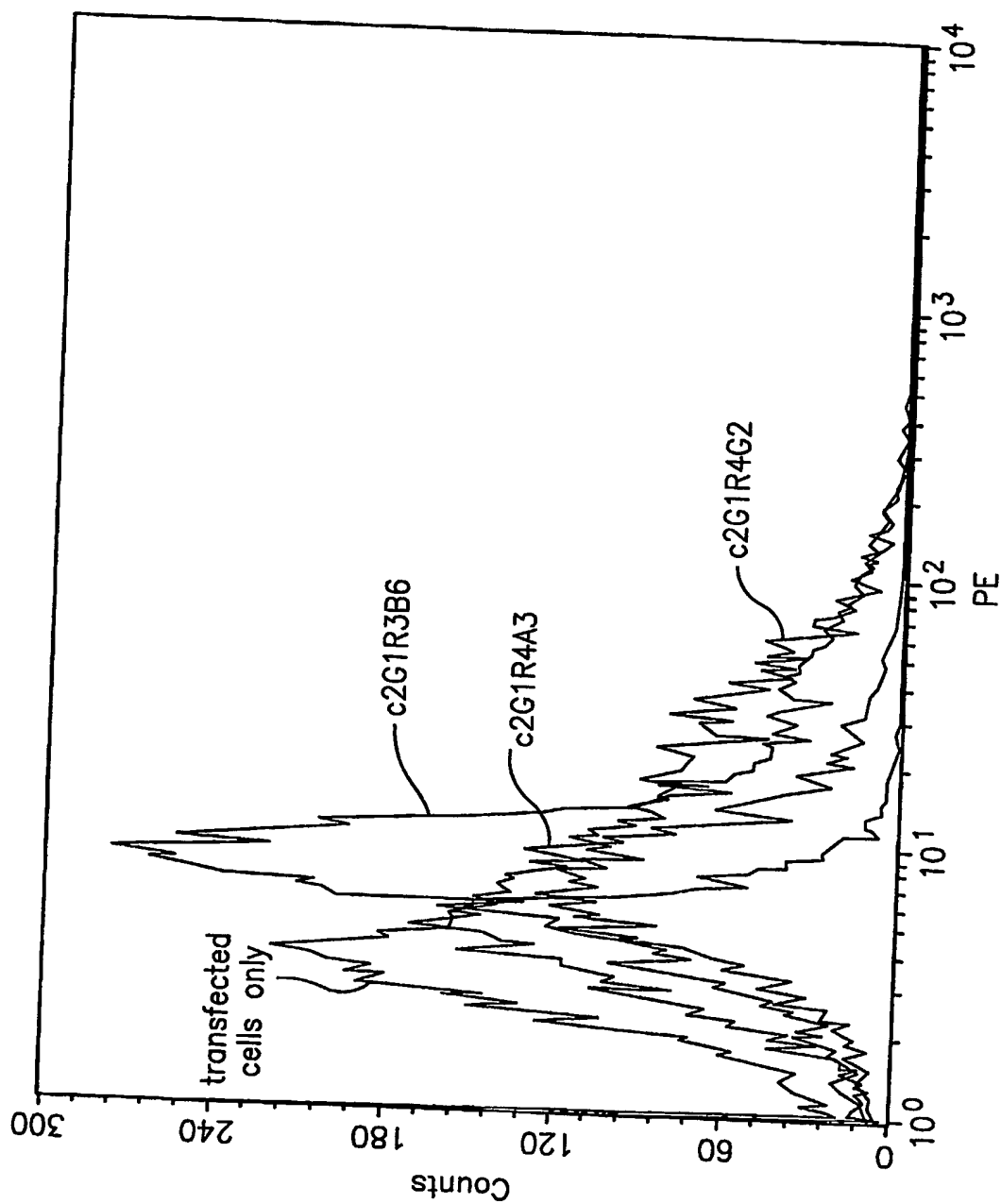


FIG. 18C

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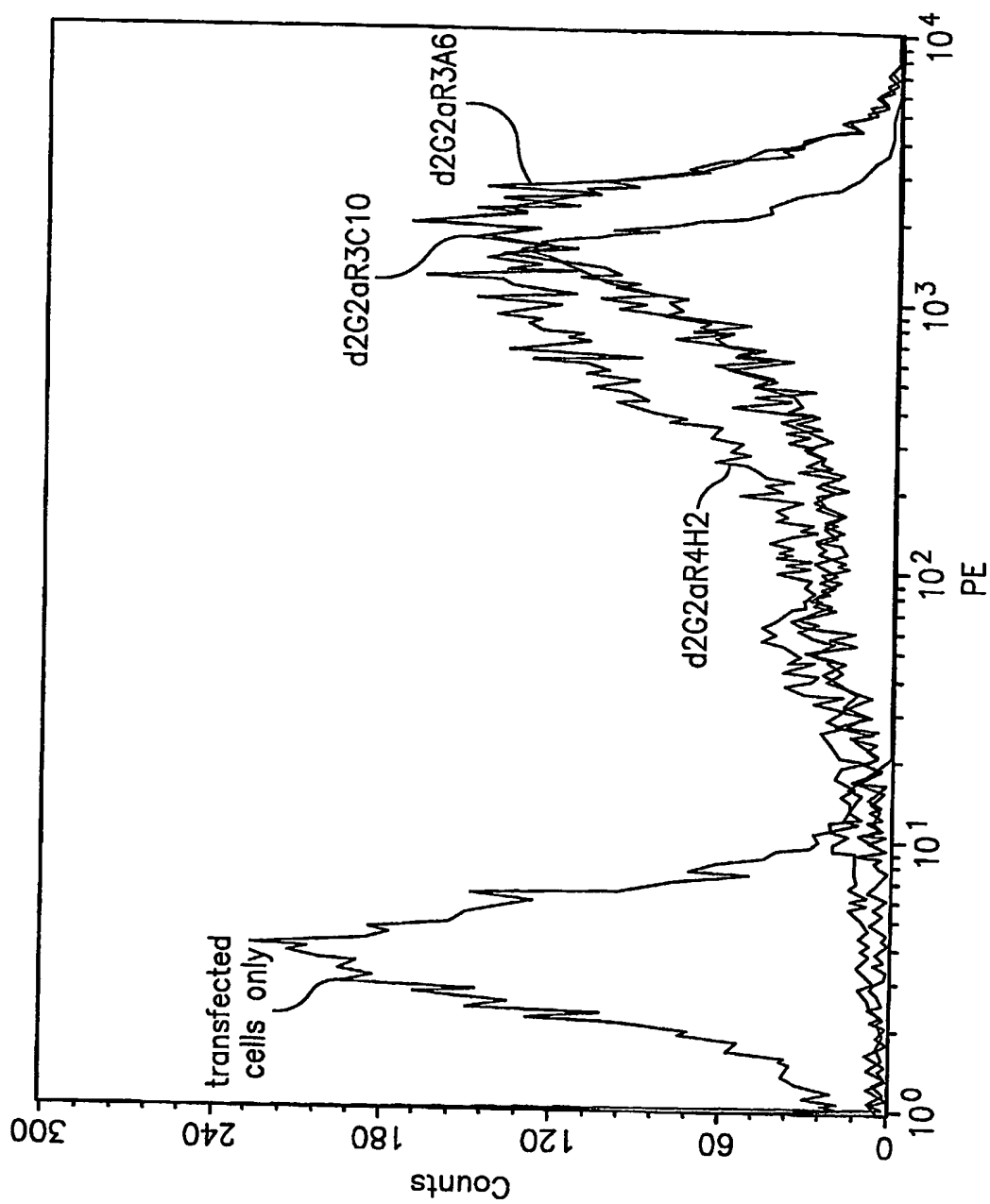


FIG. 18D

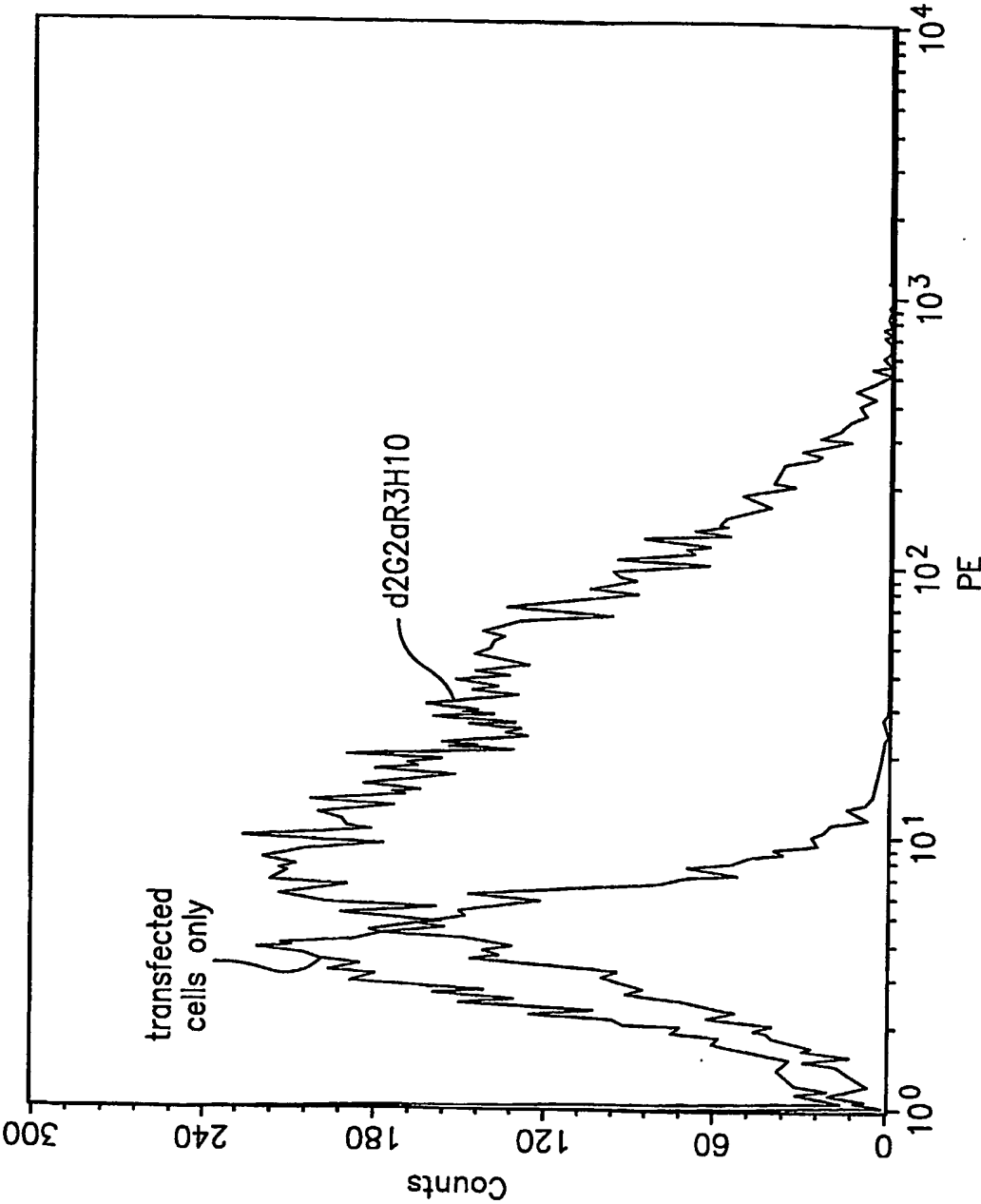


FIG. 18E

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FLJ32028 binding clones

Fab	FR1	CDR1	FR2	CDR2
c2G1R3A3	LEVQLVESGGGLVQPKGSLKLSCAAS	GFNFNTYAMN	WVRQSPGKGLEWVA	RIRTKSNNYATYYADSVKD
c2G1R3A12A.....S.....
c2G1R3C2A.....S.....
c2G1R3E3A.....S.....
c2G1R3F5	...K.....A.....S.....
c2G1R3F6	...K.....A.....S.....
d2G1R4B6A.....S.....
c2G1R4C9A.....S.....
c2G1R3B6	LEVQLQQSGAEIAKPGASVKMSCKAS	GYFTNSWIH	WVKQRPQGQGLEWIG	YIHPGPGYTEYNQNFKD
c2G1R4G2	LEVQLKQSGAEIVKPGASVKLSCTAS	GFNIKDTYMN	WVKQRPQGQGLEWIG	GIDPANDNTEYVPKFQGG
c2G1R4A3Q.....
d2G1R4B12	LEVQLQQSGAEIVRPGASVTLSCAS	GYFTDYEMH	WVKQTPVHGQLEWIG	GIDPETGGTVYNQKFKG
d2G1R4B8
d2G2aR3A6	LEVQLVESGGGLVQPKGSLKLSCAAS	GFTFNTYAMN	WVRQAPGKGLEWVA	RIRTKSNNYATYYADSVKD
d2G2aR3C10
d2G2aR4A12
d2G2aR4G6
d2G2aR4H2
d2G2aR3H10	LEVQLQQSGAEIVRPGASVTLSCAS	GYFTDYEMH	WVKQTHVHGQLEWIG	GIDPETGGTVYNQKFKG

FIG. 19

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Fab	ELISA/FACS				SEQ ID NO:
	FR3	CDR3	FR4	(geo mean)	
c2G1R3A3	RFSVSRDDSQSMLYLQMNLLKTEDTAMYYCVR	HEGDWFAY	WGQGTLLTVTSE	0.582/20.46	24
c2G1R3A12	..TI.....	0.492/16.02	25
c2G1R3C2	..TI.....	0.481/9.68	25
c2G1R3E3	..TI.....	0.507/12.67	25
c2G1R3F5	..TI.....	0.356/12.61	26
c2G1R3F6	..TI.....	0.446/14.7	26
d2G1R4B6	..TI.....	0.980/156	25
c2G1R4C9	..TI.....	..N....	0.360/24.62	27
c2G1R3B6	KATLTADKSSSTAYIQLSSLTSEDSAVYYCIR	GGDWGY	WGQGTSLTVSS	1.184/10.6	28
c2G1R4G2	RATITADTSSNTAYLQLRSLTSDDTAVYYCVT	GGYFDY	WGQGTLLTVSS	2.412/11.08	29
c2G1R4A3	0.743/5.81	30
d2G1R4B12	KATLTADKSSSTAYMELRSQTSSEDSAVYYCTR	WDY	WGQGTLLTVSS	1.426/13.8	31
d2G1R4B8	1.415/17.84	31
d2G2aR3A6	RFTISRDDSQSMLYLQMNLLKTEDTATYYCVR	QGENRFAY	WGQGTLLTVSA	0.622/552.3	32
d2G2aR3C10	0.574/535	32
d2G2aR4A12	0.592/366	32
d2G2aR4G6	0.184/164	32
d2G2aR4H2	0.551/301.8	32
d2G2aR3H10	KATLTADKSSSTAYMELRSQTSSEDSAVYYCTS	SLP	WGQGTLLTVSA	0.740/16.41	33
negative control					

Top sequence in each group is used as a reference for others in the same group. Only the amino acids different from the reference sequence are shown in each sequence and the same amino acids are indicated by dots. ELISA is OD405 reading at 60 min to the FLJ32028 coated wells (4 µg/ml) and FACS (geo mean) is showing geometric mean.

FIG. 19 (Cont.)

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